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## Effect of Transfluthrin-Impregnated Insecticide Paper on Some Biochemical Parameters and Lung Histopathology in Rats

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**Abstract** Serum malonaldehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and histological analysis of lungs were carried out in twenty rats exposed to smoke from transfluthrin impregnated insecticide paper. The rats were divided into five groups (I, II, III, IV and V) of four each. Group one served as control while Group II, III, IV and V were exposed to smoke from transfluthrin impregnated paper Insecticide for 15, 30, 60, 120 minutes daily for four weeks respectively. On the 29th day all the rats in the five groups were euthanized and blood sample was collected and centrifuged for analysis of biochemical parameters (MDA, AST, ALT and ALP), the animals were dissected and tissue from the lungs were collected for histological studies. Significant increase ( $p < 0.05$ ) in all the parameters were observed in time dependent manner when compared with control. However, histopathological analysis of the lung tissue shows no pathological changes. Thus, the chemical in the transfluthrin impregnated insecticide paper can modify biochemical parameters but within the context of duration of this research, no significant impact in lung tissue was observed. However, transfluthrin impregnated paper insecticide should be used with caution even at lower doses.

**Keywords** Biochemical, Insecticide, Pathological, smoke and Transfluthrin

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### 1. Introduction

Malaria is a disease caused by the injection into human body of plasmodium parasite due to the bite of certain species of female anopheles mosquitoes. The infective forms (sporozoites) of one or more of at least four different species of plasmodium invade the liver and subsequently red blood cells giving rise to periodic shivering, pyrexia and sweating [1]. Malaria is by far the world's most important tropical parasitic disease killing people more than any other communicable disease except AIDS and Tuberculosis. Worldwide prevalence of the disease is in the order of 350-500 million clinical cases each year, with an estimated annual death of over 1.1 million deaths each year [2-10]. Mosquitoes are common insect in the family culicidae within the order dipthera, class insecta and phylum arthropoda, there are about 3,500 species of Mosquitoes found throughout the world [11]. In some species of Mosquitoes, the females feed on human and are therefore serves as vectors for a number of infectious diseases,



human malaria is transmitted only by females of the genus *Anopheles*, they are approximately 430 *Anopheles* species only 30-40 transmit malaria (i.e are vectors) in nature. Mosquitoes go through four stages in their life cycle: larva, pupa and adult. Mosquitoes in which the female mouthparts are adapted for piercing and sucking belong to subfamily culicinae, the common house mosquito is classified as *Culex pipiens*, and the species that transmit malaria are classified in the genus *Anopheles*.

Pesticides may be applied to control larvae (larvicides) or adults (adulticides). Applications of adulticides or larvicides are made after the presence of mosquitoes has been demonstrated by surveillance procedures.

Application is made by prescribed standards. All insecticides must have the name and amount of active ingredient (AI) appearing on the label; examples are DEET and pyrethroids [12].

Pyrethroids are synthetic chemical similar to the natural chemical pyrethrins produced by the flowers of pyrethrums (*Chrysanthemum cinerariac folium* and *C. coccincum*). Pyrethroids now constitute a major proportion of the synthetic insecticide market and are common in commercial products such as household insecticide, and insect repellent in a concentration that is generally harmless to human beings in low doses but can harm sensitive individuals [13].

Insecticide paper is an efficient mosquito killer for indoor use, specially developed to combat malaria by eradication the mosquitoes; the active ingredient is pyrethroidtransfluthrin which paralyses the mosquitoes by causing their sodium channels open in the neuronal membrane.

The lungs are paired organs in the chest that perform respiration; it is an essential respiratory organ in all air breathing animals. The lungs are covered by a protective membrane called the pulmonary pleura [14]. The principal function of the lungs is to transport oxygen from the atmosphere into the blood stream and to transport carbondioxide from the bloodstream into the atmosphere [15]. This study is aimed at assessing the effect of smoke from transfluthrin impregnated insecticide paper on serum malondealdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and its histopathological effect on rat lungs.

## 2. Materials and Methods

### 2.1. Study Animals

Male Albino rats weighing 120 g were obtained from Department of Biological Sciences, Bayero University Kano. Animals were housed in colony cages at an ambient temperature and relative humidity. The animals had free access to standard palletized grower feed and drinking water. The principles of laboratory animal care and guidelines were followed.

### 2.2. Insecticide Paper

The insecticide paper used in the research is a booklet containing sheets of papers treated with transfluthrin 0.45 %, and essential oils 2.5 % as it active ingredients, each sheet is weighing 0.905 g and 16.9 cm in length and 7.2 cm in width.

### 2.3. Experimental Protocol

This research is an interventional treatment of albino rats with smoke from transfluthrin impregnated insecticide paper. Twenty (20) male albino rats were divided into five groups (I, II, III, IV and V) of four animals each, group one served as normal control while Groups (II, III, IV and V) were exposed to smoke from transfluthrin based impregnated paper Insecticide for 15, 30, 60, 120 minutes daily for 28 days respectively.

On the 29<sup>th</sup> day, all the rats were euthanized and blood sample was collected in a dried centrifuge tube for biochemical analysis the carcasses were dissected and from the lungs tissue were collected for histological studies. Aspartate aminotransferase (AST) and alanine aminotransferases (ALT) activity were assayed using Reitman and Frankel method [16], alkaline phosphatase (ALP) activity was assayed using the method developed by Roy [17], serum malondialdehyde was estimated using Ohkawa *et al* [18] method while the histopathological analysis was done by staining technique according to Auwioro [19].



## 2.4. Statistical Analysis

All data were expressed as mean  $\pm$  standard deviation. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test after investigating the data for normality using Shapiro-Wilk test and for variances homogeneity to be sure that the data are normally distributed and variances would be homogenous using GraphPad Instat3 Software version 3.05. Differences of  $P < 0.05$  were considered to be significant.

## 3. Result and Discussion

### 3.1. Result

Table 1 present the effect of exposure to graded dosage transfluthrin based insecticide paper on MDA, ALP, ALT and AST concentrations in experimental rats, there is a significant increase in all parameters (MDA, ALP, ALT and AST) in test groups (group II, III, IV and V) compared to normal control (group I) except for ALP and AST which only shows a remarkable increase in groups (III, IV and V). While plate (1-5) shows the histopathological Analysis of lung tissue from experimental rats exposed to graded doses with transfluthrin based paper Insecticide.

**Table 1:** Effect of Transfluthrin based Insecticide Paper on MDA, ALP, ALT and AST concentrations in experimental rats.

Groups	Time of exposure (min)	MDA (nmol/ml)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
I	-	0.5 $\pm$ 0.17 <sup>a,b,c,d</sup>	43.75 $\pm$ 2.04 <sup>a,b,c</sup>	9.50 $\pm$ 1.30 <sup>a,b,c</sup>	8.50 $\pm$ 2.40 <sup>a,b,c,d</sup>
II	15	0.9 $\pm$ 0.20 <sup>a</sup>	55.78 $\pm$ 2.78	18.25 $\pm$ 2.75	20.28 $\pm$ 4.30 <sup>a</sup>
III	30	1.2 $\pm$ 0.30 <sup>b</sup>	86.20 $\pm$ 3.56 <sup>a</sup>	22.25 $\pm$ 2.08 <sup>a</sup>	23.75 $\pm$ 3.30 <sup>b</sup>
IV	60	1.4 $\pm$ 0.17 <sup>c</sup>	127.00 $\pm$ 3.80 <sup>b</sup>	24.25 $\pm$ 2.22 <sup>b</sup>	24.25 $\pm$ 3.00 <sup>c</sup>
V	120	1.5 $\pm$ 0.13 <sup>d</sup>	126.17 $\pm$ 4.18 <sup>c</sup>	26.50 $\pm$ 3.10 <sup>c</sup>	29.25 $\pm$ 2.60 <sup>d</sup>

Results are presented as Mean  $\pm$  standard deviation, (n=4). Values with the same superscripts in a column are significantly different compared to each other ( $P < 0.05$ )

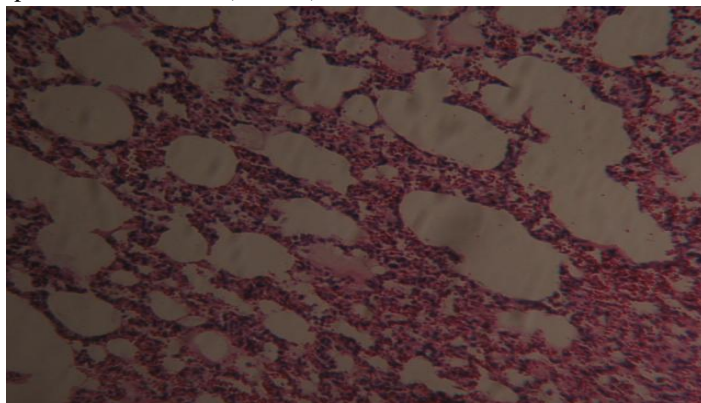


Plate 1: Section of lungs of control rat showing no pathological changes (H and E, mag.  $\times 100$ )

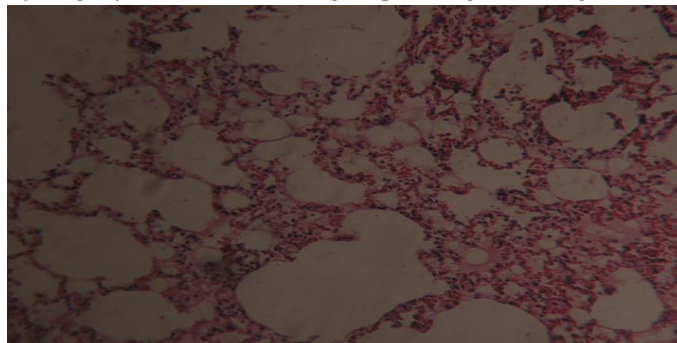
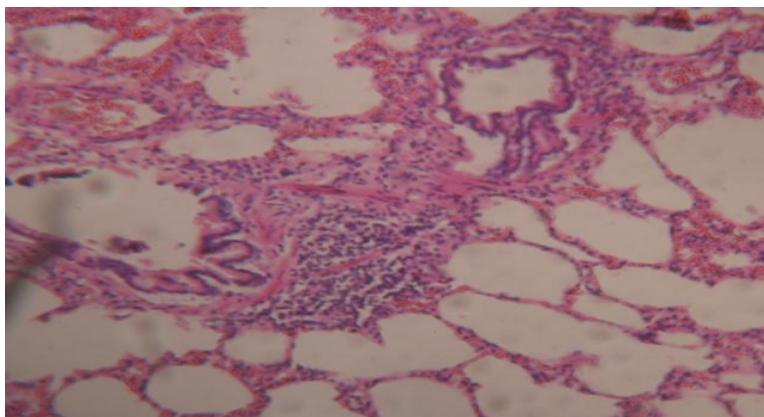
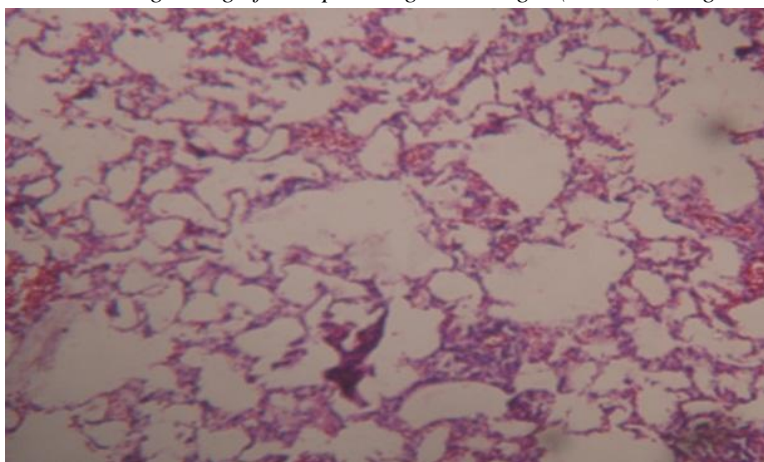


Plate 2: Section of rat lungs exposed to smoke from transfluthrin based impregnated paper Insecticide for 15 minutes showing no significant pathological changes (H and E, mag.  $\times 100$ )

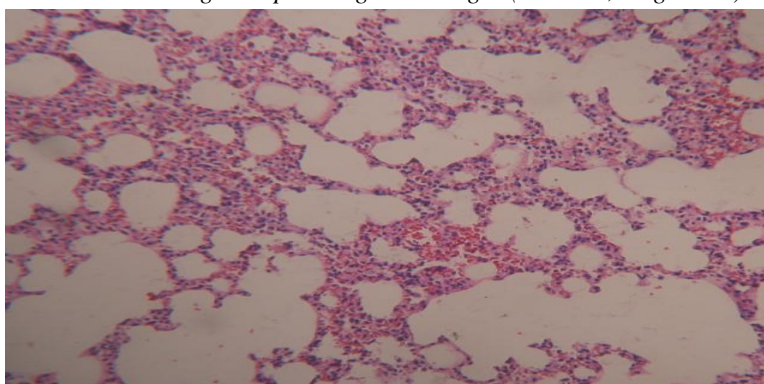




*Plate 3: Section of rat lungs exposed to smoke from transfluthrin based impregnated paper Insecticide for 30 minutes showing no significant pathological changes (H and E, mag.  $\times 100$ )*



*Plate 4: Section of rat lungs exposed to smoke from transfluthrin based impregnated paper Insecticide for 60 minutes showing mild pathological changes (H and E, mag.  $\times 100$ )*



*Plate 5: Section of rat lungs exposed to smoke from transfluthrin based impregnated paper Insecticide for 60 minutes showing mild changes (H and E, mag.  $\times 100$ )*

### 3.2. Discussion

Liver damage is a serious disease characterized by disturbances in the normal functions of the liver. It is clinically diagnosed by determining the serum concentration of liver enzymes (ALT, AST and ALP). ALT and AST are non-plasma specific enzymes involved in transamination of aspartic acid and alanine respectively, the enzymes were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver





as in viral or toxic hepatitis [21]. ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline PH. These enzymes were reported to reach higher than normal level in the blood in events of impaired liver function [22]. Thus, they are used as serum markers of hepatic damage. Malondialdehyde is used as a measure of lipid peroxidation. Lipid peroxidation is a chain of reaction providing a continuous supply of free radical that initiates further peroxidation [23]. Malondialdehyde is used to determine the indices of oxidative stress in the serum. in this research, significant increase in the level of MDA in transfluthrin impregnated paper insecticide treated groups (II, III, IV and V) was observed compared to the normal control G I this shows that reactive oxygen species were produced when the rats were expose to doses of transfluthrin impregnated paper insecticide which indicate that there is oxidative stress that depends upon the time of exposure.

A significant increase ( $p < 0.05$ ) in serum levels of ALT, AST and ALP was observed in groups (II, III, IV and V) compared to the controls (Group I), this an indication of induction of liver damage caused by the insecticide paper. The enzymes were reported to be higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis [24]. This finding support the result of a research carried by Ray [25] who reported that preteroids are generally harmless to human beings in low doses but can harm sensitive individuals. However, no pesticide is 100 percent safe and care must be exercised in the use of any pesticide [27]. The histopathological examination of lungs shows normal cellular architecture with no significant difference between groups. This may be due to the fact that effects in tissue are produce after a prolonged frequent exposure.

#### 4. Ethical Approval

All authors hereby declare that Principle of laboratory animal care [28] and ethical guidelines for investigation of experimental pain in conscious animals [29] were observed during experimentation

#### 5. Conclusion

It can be concluded that within the context of time and dose used in the research, exposure to doses of transfluthrin impregnated paper insecticide lead to liver damage and oxidative stress but does not lead to lungs failure. However, it can be toxic on long time exposure even at lower concentration therefore it should be used with great caution

#### References

1. World Health Organization (2015). World Malaria Report, Geneva, Switzerland. 11-15
2. Bruce-Chwatt, L.J. (1988). *Principles and Practice of Malariology*. In Wernsdorfer W. and McGregor 2<sup>nd</sup> edn Churchill Livingstone, London. 20-59.
3. World Health Organization (2015). World Malaria Report, Geneva Switzerland. 11-15
4. World Health Organization (2006). Department of Immunization, Vaccines and Biologicals. State of the art of the new vaccine and development, Geneva, Switzerland 48-56. [www.who.int/vaccines-documents/](http://www.who.int/vaccines-documents/)
5. Trager, W. and Jensen, J.B. (1976). Human Malaria Parasites In continuous Culture. *Science*. 193:673-675.
6. Shortt, H.E, Garnham, P.C., Covell, G. and Shute, P.G. (1948). The Pre-Erythrocytic Stage of Human Malaria Plasmodium Vivax, *British Medical Journal*. 1(4550):547.
7. Shortt, H.E, Garnham, P.C., Covell, G. and Shute, P.G. (1948). The Pre-Erythrocytic Stage of Human Malaria Plasmodium Vivax, *British Medical Journal*. 1(4550):547.
8. Hempelmanne, T. I. and Ksyn, B.J. (2009). "Kurzgefasste Geschi Chteder Malaria – Chemotherapic Von Zwiebelnbis Zum Artemisinin" *Pharm Unserer Zeit*. 38(6):500-507.
9. Center for Disease Control and Prevention. (2015). Treatment of Malaria: Guidelines for Clinicians (United States): Part 2: General Approach to Treatment and Treatment of Uncomplicated Malaria. 23-30.
10. McGregor. (1996). *The Wellcome Trust Illustrated History of Tropical Diseases*, 5<sup>th</sup>edn. Wellcome Trust London. 230-247.
11. Leopoldo, M. R., (2008). Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Hydrobiologia*. 595(1): 477-487.



12. American mosquito control Association. (2014). Control of mosquitos, retrieved on 4<sup>th</sup> august 2016. <http://www.mosquito.org/control>
13. Ray, D.E. (1991). *Pesticides derived from plants and other organisms*. In: *Handbook of Pesticide Toxicology*. Academic Press, Toronto. 585-593.
14. Fischer, H. (2009). Mechanism and Function of Duxin epithelia of the lung. *Antioxidants and redox signaling*. 11(10):2453-65.
15. Stedman's Medical Dictionary. 2006.
16. Reitman, S. and Frankel S. (1957). A Colorimetric Method for the Determination of Serum Glutamate-Oxaloacetate and Pyruvate Transaminase. *American Journal of Clinical Pathology*. 28:56.
17. Roy, A.V. (1970). Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein Monophosphate. *Clinical Chemistry*. 16:431.
18. Ohkwa, H., Ohishi, N. and Yagi, K. (1979). Assay for Lipid Peroxide in Animal Tissues by Thiobarturic Acid Reaction. *Anal of Biochemistry*. 95:351-8.
19. Auwioro, O.G. (2010). *Histochemistry and tissue pathology: Principles and techniques*. 2<sup>nd</sup> edition, University press delta state university, Abraka Nigeria. 561-568.
20. GraphPad Instat3 Software (2000). Available: [www.graphpad.com](http://www.graphpad.com). Retrieve on 23<sup>rd</sup> February 2015.
21. Debruin A. (1976) Biochemical Function Test. In *Biochemical Toxicology of Environmental Agents*. *Elsvier*. 451-470.
22. Sule, M.S. and Abubakar S.M. (2004). Effect of Oral administration of graded doses of aqueous extract of *Cassia Occidentalis*. *Biol. Env.Sc.J. For the Tropics*. 5(2):41-55.
23. Price, N.C. and Stevens, L.(2003) *Fundamentals of Enzymology*. 3<sup>rd</sup> edition. Oxford University Press, Oxford. 404-406.
24. Ghosh, J., Das, J., Manna, P. and Sil, P.C.(2010). Acetaminophen induced renal injury via oxidative stress and TNF- $\alpha$  production: therapeutic potential of arjunolic acid. *Toxicology*. 268: 8-18.
25. Muhammad, I.U., Alhassan, A.J., Wudil, A.M. and Jarumi, I.K. (2015). Toxicological and Protective Effect of Aqueous Stem Bark Extract of *Khaya senegalensis* (ASBEKS) on Liver of Experimental Rat. *British Journal of Applied Science & Technology* 9(6): 600-605.
26. Ray, D.E. (1991). *Pesticides derived from plants and other organisms*. In: *Handbook of Pesticide Toxicology*. Academic Press, Toronto. 585-593.
27. American mosquito control Association. (2014). Control of mosquitos, retrieved on 4<sup>th</sup> august 2016. <http://www.mosquito.org/control>
28. NIH. (1996) Guidelines for the care and use of laboratory animals. National Academic Press, NIH Publication. 85: 23.
29. Zimmermann, M. (1983). Ethical guidelines for investigation of experimental pain in unconscious animals. *Pain*. 19:109-110.

